Associational effects and the maintenance of polymorphism in plant defense against herbivores: review and evidence

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Abstract

Many plant species have evolved defense traits against herbivores. *Associational effects* (AE) refer to a kind of apparent interaction where the herbivory risk to a focal plant species depends on the composition of other plant species in a neighborhood. Despite ample evidence for AE between different plant species, this point of view has rarely been applied to polymorphism in defense traits within a plant species. The purpose of this review is to highlight an overlooked role of conspecific AE in maintaining polymorphism in antiherbivore defense. First, I present a general review of AE between plant species and its role in the coexistence of plant species. This viewpoint of AE can be applied to genetic polymorphism within a plant species, as it causes frequency- and density-dependent herbivory between multiple plant types. Second, I introduce a case study of conspecific AE in the trichome-producing (hairy) and glabrous plants of *Arabidopsis halleri* subsp. *gemmifera*. Laboratory and semi-field experiment illustrated that AE against the brassica leaf beetle *Phaedon brassicae* mediate a minority advantage in defense and fitness between hairy and glabrous plants. Combined with a statistical modeling approach, field observation revealed that conspecific AE can maintain the trichome dimorphism via negative frequency-dependent selection in a plant population. Finally, I discuss spatial and temporal scales at which AE contribute to shaping genetic variation in antiherbivore defense in a plant metapopulation. Based on the review and evidence, I suggest that AE play a key role in the maintenance of genetic variation within a plant species.

Keywords: Frequency-dependent selection, Genetic variation, Herbivory, Neighbor effects, Plant defense

1. Background

Many plant species have evolved defensive traits, such as spines and toxins, against herbivores (Schoonhoven *et al*. 2005). Natural plant populations exhibit genetic variation in regard to chemical (Hughes 1991; van Dam *et al*. 1999; Chan *et al*. 2010) and morphological defenses (Kivimäki *et al*. 2007; Wise *et al*. 2009). It is well known that these defense traits are costly for plant growth and/or reproduction (e.g., Mauricio 1998; Elle *et al*. 1999; Züst *et al*. 2011). The theory of plant defense assumes that the defense–growth tradeoff favors an optimal balance between the cost and benefit of defense (Simms 1992), extinguishing variation in defense levels. Thus, it is difficult for natural selection to maintain genetic variation in antiherbivore defense traits unless a stabilizing mechanism occurs.

Plant defense theory has focused on how individual plants allocate their resource to defensive traits (e.g., Rhoades 1979; Simms 1992; Stamp 2003). However, the defensive effects of a particular trait depend not only on a plant's own trait but also on the composition of the other plant types in a neighborhood (Agrawal *et al*. 2006; Barbosa *et al*. 2009; Underwood *et al*. 2014). These phenomena are called *associational effects* (AEs: also known as *neighbor effects*), which occur when "consumer effects on individuals of one resource organism type, at a given density of that type, are a function of the neighborhood composition of other resource types at particular spatial scales" (Underwood *et al*. 2014). An increasing number of studies have shown the importance of AE in maintaining plant species diversity (e.g., Hay 1986; Callaway *et al*. 2005; Stastny & Agrawal 2014), but this point of view has rarely been tested in the evolutionary ecology of antiherbivore defenses.

The purpose of the present review is to highlight the overlooked but potentially

critical role of conspecific AE in the maintenance of genetic variation in antiherbivore defense traits. This paper consists of three chapters, in which I first outline the ecological roles of AE in maintaining plant species diversity. Second, I introduce a case study on *Arabidopsis* trichomes to expand the concept of AE to polymorphism in defense within a plant species. Finally, I discuss how AE contributes to intraspecific variation in plant defense at larger spatial and temporal scales. Based on the review and evidence, the present paper suggests that AE plays a key role in maintaining not only species diversity but also genetic variation in antiherbivore defense.

1-1. Examples of AE in plant-herbivore interaction

The phenomena of associational resistance or susceptibility have been reported across agricultural (Tahvanainen & Root 1972; Risch 1981; Le Guigo *et al*. 2012), forest (White & Whitham 2000; Sholes 2008; Castagneyrol *et al*. 2013), grassland (Agrawal 2004; Courant & Fortin 2010; Hahn & Orrock 2016), semi-arid (González-Teuber & Gianoli 2007), wetland (Rand 2003; Hughes 2012), and aquatic (Hay 1986; Wahl & Hay 1995) ecosystems (see Appendix for selected examples). Two major patterns have been reported for AE between plant species: Associational *resistance* or *susceptibility* refer, respectively, to a situation where a focal plant species is *less* or *more* damaged in the presence of another plant species. Undefended plants may gain “associational resistance” when the defended plants are protected from herbivory by nearby plants (Tahvanainen & Root 1972; Rausher 1981; Hambäck *et al*. 2000). In the polyculture of the collard green *Brassica oleracea* var. *acephala* (Brassicaceae), Root and colleagues for the first time developed the concept of “associational resistance” and “resource concentration,” which refer to the effects of vegetation composition and total density on

herbivore populations, respectively (Tahvanainen & Root 1972; Root 1973). Recently, it was also discovered that neighboring plants do not always reduce herbivory damage to other plant species. The term “associational susceptibility” was coined to refer to the situation where defended plants suffer from severe herbivory when they occur nearby undefended ones (White & Whitham 2000; Rand 2003). Barbosa *et al*. (2009) introduce many more examples of AE and Rautio *et al*. (2012) outline the terminology regarding AE, comprising the terms “plant defense guild” (Atsatt & O’Dowd 1976), “shared doom” (Wahl & Hay 1995; Emerson *et al*. 2012), and “associational refuge” (Hjältén *et al*. 1993; Miller *et al*. 2009; Emerson *et al*. 2012; Hughes 2012).

The mechanisms underlying AE are involved in various combinations of plant traits and herbivore behaviors. In general, herbivores alter their preferences and/or performance depending on their dietary menu (e.g., Lefcheck *et al*. 2013; Sato & Kudoh 2016a; Wetzel *et al*. 2016). In particular, theory of optimal foraging suggests that consumers should utilize suboptimal resource as the density of optimal resource becomes low (e.g., Charnov 1976). This behavioral basis of consumers directly alters the consumption rate and thus drives apparent competition between optimal and suboptimal resource (Holt & Kotler 1987). Empirically, mammalian herbivores often avoid spiny or toxic herbs and thereby lead these defended herbs to confer associational resistance to undefended plant species at a patch level (e.g., Hjältén *et al*. 1993; Callaway *et al*. 2005; Courant & Fortin 2010; Miller *et al*. 2009). Visual or chemical crypsis conferred by neighboring plants results in associational resistance against insect herbivores (Tahvanainen & Root 1972; Rausher 1981; Hambäck *et al*. 2000). Volatile organic chemicals from neighboring plants sometimes induce plant resistance traits and thus drive associational resistance against herbivores (Karban & Maron 2002; Karban

2007; Zakir *et al*. 2013). Direct competition sometimes increases plants' investment in defense traits and may promote associational resistance (Barton & Brower 2006; González-Teuber & Gianoli 2007). Polyphagous herbivores move from primary to secondary host species during their dispersal process, resulting in associational susceptibility on the part of the former to the latter host plant (White & Whitham 2000; Agrawal 2004). These examples suggest that the outcomes of AE depend on herbivore behaviors and plant trait changes.

While long recognized as apparent competition, the outcomes of AE are more complex than simple competition between two plant species. AE comprise facilitation, exploitation, and other kinds of species interaction (Agrawal 2004; Barbosa *et al*. 2009). In particular, recent reviews pointed out problems with the terminologies and experimental designs used to test AE (Rautio *et al*. 2012; Underwood *et al*. 2014), as there has been little consensus regarding the trait of a focal plant type (i.e., defended or undefended) and manipulations on the neighborhood composition (presence/absence, relative abundance, or total density of multiple plant types) (see also Appendix). For example, a number of studies compared the magnitude of herbivory on one of two plant species between the presence and absence of another species (Hambäck *et al*. 2000; White & Whitham 2000); however, this setting is unable to determine the likelihood of the coexistence of two plant species (Underwood *et al*. 2014). Many studies focused on the absolute amount of herbivory (Tahvanainen & Root 1972; Hambäck *et al*. 2000; White & Whitham 2000), while others compared the relative amounts of herbivory between multiple plant types (Bergvall *et al*. 2006; Castagneyrol *et al*. 2013). Furthermore, some studies focused on the herbivory load to individual plants within a small spatial scale (referred to as a "patch": Rausher 1981; Hambäck *et al*. 2000;

145 Karban & Maron 2002) while others compared herbivory among the plant patches at
146 broader spatial scales (Bergvall *et al*. 2006; Castagneyrol *et al*. 2013). Details of the
147 experimental design and outcomes are listed for 16 publications (see Appendix).
148
149 *1-2. Frequency/density-dependent damage caused by AE*
150 Recently, several authors have begun to discuss how AE enable the coexistence of
151 multiple plant species. Underwood *et al*. (2014) advocated that the focus should be on
152 the frequency- or density-dependent damage caused by AE to multiple plant species. If
153 AE is more likely to protect rare plant species than abundant species, this rarity
154 advantage (i.e., negative frequency dependence) in defense would prevent rare species
155 from going extinct due to herbivory, thereby promoting the coexistence of multiple
156 plant species. In this refined framework, negative or positive frequency-dependent
157 damage indicates associational resistance or susceptibility for rare plant species,
158 respectively. Even if multiple plant species can coexist via AE, recovery from low
159 density (i.e., negative density dependence) is necessary for the long-term persistence of
160 plant populations. The negative or positive density-dependent damage refers to the
161 “resource concentration or delusion” hypothesis, initially developed by Root (1973) and
162 recently reviewed by Underwood *et al*. (2014) and Hambäck *et al*. (2014).
163 Several studies suggest the importance of plant frequency and density in
164 determining the outcome of AE, although those showing the importance of both the
165 density and frequency dependence are still limited (Table 1). Kim and Underwood
166 (2015) manipulated the density and frequency of the tall goldenrod *Solidago altissima*
167 (Asteraceae) and the Carolina horsenettle *Solanum carolinense* (Solanaceae). As a result,
168 *S. carolinense* received more damage due to the combined effects of the high

conspecific density and intermediate frequency of the two species (Kim & Underwood 2015). Hahn and Orrock (2016) manipulated both the density and frequency of two related species *Solidago nemoralis* and *S. odora* (Asteraceae) in the field and then revealed herbivore foraging to be a key mechanism with a behavioral assay. The less defended species *S. nemoralis* gained associational resistance when it was rare due to the reduced foraging activity of grasshoppers, with the two *Solidago* species more likely attacked at a higher density (Hahn & Orrock 2016). In relation to herbivore foraging, Verschut *et al*. (2016) showed that low-concentration of balsamic vinegar was less likely to attract the fruit fly *Drosophila melanogaster* at a high frequency of high concentration diets, while high-concentration diets were more likely to attract flies at a high frequency of low-concentration diets. Previous studies on the fallow deer *Dama dama* manipulated the tannin concentration as well as the frequencies of two resource types (Bergvall & Leimar 2005; Bergvall *et al*. 2006). High tannin diets were consumed less frequently when low tannin diets were abundant, whereas low tannin diets were consumed more frequently when high tannin diets were abundant (Bergvall & Leimar 2005). Furthermore, if two species co-occur in their natural habitats, field surveys help us capture the effects of plant density or frequency in the wild population. Russell and Louda (2005) documented that the native thistle *Cirsium undlatum* (Asteraceae) received less florivory from the exotic weevil *Rhinocyllus conicus* as the number of another native thistle (*C. canescens*) increased in close vicinity. This long-term survey found that an invasive herbivore creates a novel AE due to the overlap of flowering phenology between two native plant species (Russell & Louda 2004; Russell & Louda 2005).

Although density- or frequency-dependent herbivory have been shown

experimentally, we still know little about how AE occurs and contributes to the maintenance of plant species diversity. Thus, it is necessary to link the mechanisms and patterns of AE in a single study system. There is a common set of empirical approaches for understanding the mechanisms and consequences of AE from laboratory to the field study (Fig. 1: see also Appendix for details). The first step is to discover candidate mechanisms of AE (Fig. 1), including the identification of key plant traits and herbivore behaviors, and then to address how they cause non-additive herbivory between multiple plant species. Previous studies have done this by conducting laboratory choice experiments (e.g., Tahvanainen & Root 1972; White & Whitham 2000; Rand 2003) or by observing herbivore movements between plants (Rausher 1981; Bergvall *et al*. 2006). The second step includes experimental tests to determine whether plant damage and/or fitness depend on the neighborhood composition. This step is adopted by almost all the studies and is the most important for illustrating the relevance of AE. The third step is multi-year field survey to corroborate the experimental evidence with field observations on plant damage and population dynamics across years (Fig. 1). This final step is executable if the focal plant species co-occur within natural populations (Russell & Louda 2004; Callaway *et al*. 2005). These comprehensive tests from laboratory experiment to field observations will reveal the causes and consequences of AE in a single study system.

1-3. Plant defense polymorphism via the lens of conspecific AE

While the concept of AE was originally developed in interspecific interactions (Tahvanainen & Root 1972; Root 1973), it can be applied to genetic variation within the same plant species. In agricultural ecosystems, polyculture has been carried out not only

for different species but also for different cultivars within the same plant species (e.g., Cantero & Sanford 1984; Hambäck *et al*. 2009). The emerging framework of "community genetics" may be considered a kind of conspecific AE (Hughes *et al*. 2008), as these studies have shown that the polyculture of different genotypes alters herbivore abundance (e.g., Crutsinger *et al*. 2006; Johnson *et al*. 2006; Parker *et al*. 2010). Studies on community genetics also revealed that plant genetic diversity altered the strength of selection on plant genotypes via changes in plant–herbivore interaction (e.g., Johnson *et al*. 2006; Lankau & Kliebenstein 2009; Parker *et al*. 2010), although the way in which frequency-dependent selection occurs between plant genotypes remains to be explored.

Evolutionary game theory provides a logical basis for the roles of AE in maintaining polymorphism in defense levels (Sabelis & de Jong 1988; Augner *et al*. 1991; Till-Bottraud & Gouyon 1992; Tuomi *et al*. 1999). Theoretical studies analyzed conditions under which the benefits from AE and the intrinsic cost of defense allow defended and undefended plants to coexist. In clonal plants, for example, Till-Bottraud and Gouyon (1992) predicted that herbivore preference and the cost of defense lead to the evolutionary stable strategy of producing cyanogenic and acyanogenic clones at an intermediate frequency. Empirically, this hypothesis regarding evolutionary stability can be tested by asking whether or not rarer plant types have higher fitness compared to abundant ones under AE (reviewed by Rautio *et al*. 2012). If a rarity fitness advantage reciprocally occurs among multiple plant genotypes, AE leads to negative frequency-dependent selection that can prevent rare genotypes from going extinct (Clarke 1964; Ayala & Campbell 1974). This criterion would allow empirical researchers to test whether AE can be a mechanism maintaining polymorphism in antiherbivore defense.

Despite its logical basis, only a few studies have focused on conspecific AE with regard to the maintenance of polymorphism in antiherbivore defense (Table 1). Wise *et al*. (2009) tested AE in the tall goldenrod *Solidago altissima*, which possesses a genetically based phenotype of ducking stem (called the "candy-cane" morph). This candy-cane morph occurs with a rare frequency but across natural populations of *S. altissima* (Wise 2009). Wise *et al*. (2009) conducted a manipulative experiment with two contrasting frequencies of candy-cane and erect-stem morphs (Table 1). The tall goldenrod received less damage from the specialist gall fly *Eurosta solidaginis* when the candy-cane morphs were abundant; however, the damage was frequency independent, as shown by the lack of a significant morph-by-frequency interaction (Wise *et al*. 2009). Recently, Garrido *et al*. (2016) tested frequency-dependent selection between tolerant (i.e., defense mitigating negative effects of herbivory on plant fitness) and resistant (defense directly reducing herbivore attacks) genotypes in the jimson weed *Datura stramonium* (Solanaceae), but found a rarity "disadvantage" against the maintenance of defense polymorphism. Thus, it remains unknown whether conspecific AE can be a mechanism for the maintenance of defense dimorphism. My collaborators and I have investigated AE against a leaf beetle in the trichome dimorphism of *Arabidopsis halleri* subsp. *gemmifera* (Brassicaceae). In the next chapter, I will introduce an example of conspecific AE and its stabilizing effect on a plant defense polymorphism.

2. Evidence

Several species of the genus *Arabidopsis* exhibit a dimorphism of trichome-producing (hairy) and trichomeless (glabrous) plants. The glabrousness of *Arabidopsis* species is

associated with the loss of function of the key gene of trichome development, *GLABRA1* (*GL1* also known as *GLABROUS1*) (Hauser *et al*. 2001; Kivimäki *et al*. 2007; Kawagoe *et al*. 2011; Bloomer *et al*. 2012). Because *GL1* is a transcriptional factor gene for the initiation of trichome development, the loss of function of this gene results in distinct phenotypes of hairy and glabrous plants (Oppenheimer *et al*. 1991; Hülskamp 2004; Ishida *et al*. 2008). The glabrous phenotype is recessive to the hairy ones, and these phenotypes are inherited in the Mendelian fashion (Kärkkäinen & Ågren 2002). Laboratory studies and field surveys have shown that hairy plants incurred less herbivory by insect herbivores compared to glabrous plants (Handley *et al*. 2005; Sletvold *et al*. 2010; Løe *et al*. 2007; Kivimäki *et al*. 2007), while trichomes impose a fitness cost on plants (Mauricio 1998; Sletvold *et al*. 2010; Züst *et al*. 2011). Owing to the visible and discrete phenotypes with the antiherbivore function, *Arabidopsis* trichomes provide an excellent system to investigate AE against herbivores within a same-plant species.

Likewise, *Arabidopsis halleri* subsp. *gemmifera* (abbreviated hereafter as *A. halleri*) have a dimorphism of hairy and glabrous plants (Fig. 2a). Hairy plants have non-glandular trichomes on their leaves and stems, whereas glabrous plants produce no trichomes, except on their stem and leaf margin. This presence/absence of leaf trichomes is associated with the allelic status of a *GL1* orthologue (Kawagoe *et al*. 2011). Hairy plants showed a lower fecundity and slower growth than glabrous ones did in the absence of herbivores (Kawagoe *et al*. 2011; Sato & Kudoh 2016b; Sato & Kudoh 2017b), indicating a fitness cost of the trichome production. Further, trichome production is unlinked with glucosinolate profiles (Sato *et al*. 2014), which may act as a chemical defense for Brassicaceae (e.g., Züst *et al*. 2012).

289 *Arabidopsis halleri* subsp. *gemmifera* is a self-incompatible perennial herb
290 distributed across Japan and the Russian Far East. Plants bloom from early April to May
291 at the lowland of Japan (Kawagoe & Kudoh 2010). Flowers are pollinated by hoverflies
292 and solitary bees. Seeds are dispersed by gravity. After flowering, plants produce clonal
293 rosettes on the main and lateral meristems (Sato & Kudoh 2017b), wherein late-spring
294 herbivory can directly affect the sexual and asexual reproduction. *Arabidopsis halleri*
295 subsp. *gemmifera* is attacked by multiple species of herbivorous insects including
296 beetles, butterflies, and sawflies from late-spring to early-summer (Sato & Kudoh 2015;
297 Sato & Kudoh 2017a). Among natural populations of *A. halleri*, the brassica leaf beetle,
298 *Phaedon brassicae* (Fig. 2a), occasionally outbreaks and impacts fruit production by
299 heavily infesting flowering stems (Kawagoe & Kudoh 2010).
300 In this second chapter, I introduce a case study of conspecific AE against the
301 leaf beetle on the trichome dimorphism of *A. halleri*. This study consists of three
302 specific approaches (Fig. 2). The first stage is to identify candidate mechanisms of
303 frequency-dependent damage between hairy and glabrous plants. In the second stage,
304 manipulative experiments are conducted to test fitness consequence and to address
305 whether AE against the leaf beetle results in negative frequency-dependent selection
306 between the two morphs. The final step is to link the experimental findings with field
307 observations by modeling mechanisms that govern plant population dynamics. These
308 three steps will provide comprehensive evidence that AE play a stabilizing role in plant
309 defense polymorphism.
310
311 *2-1. Candidate mechanisms of AE*
312 Behavioral response of herbivores to multiple resources can be a mechanism of AE (e.g.,

313 Bergvall *et al*. 2006; Verschut *et al*. 2016; Hahn & Orrock 2016). Choice assays are
314 often conducted to test the candidate mechanism of AE (Tahvanainen & Root 1972;
315 White & Whitham 2000; Rand 2003; Hahn & Orrock 2016). Several studies
316 documented that the feeding preferences of herbivores depend on the presence or
317 relative frequencies of multiple resource types (Chandra & Williams 1983; Cottam
318 1985; Behmer *et al*. 2001; Bergvall & Leimar 2005; Janz *et al*. 2005), but evidence
319 regarding such a frequency-dependent herbivory on natural variation in a plant defense
320 trait is limited.

321 My collaborators and I have conducted a four-way choice experiment
322 manipulating frequencies of hairy and glabrous leaf discs (Fig. 2b: Sato *et al*. 2014).
323 Adults of the brassica leaf beetle *P. brassicae* avoided hairy leaves when hairy leaves
324 were rare, whereas the adults did not show a feeding preference when hairy leaves
325 became abundant (Sato *et al*. 2014). The adult preference for glabrous leaves was also
326 observed in a binary choice experiment using a *gl1* mutant of the model plant species
327 *Arabidopsis thaliana* (Sato 2016). This result from a single-gene mutant provides strong
328 proof for the resistance function of the *GL1* gene against *P. brassicae*. Furthermore,
329 when adult beetles were starved or preconditioned with glabrous diets, they still
330 preferred glabrous plants (Sato & Kudoh 2016b). Contrarily, when adults were
331 preconditioned with hairy diets, this treatment weakened their feeding preference for
332 glabrous diets (Sato & Kudoh 2016b). The circumstantial evidence suggests that
333 sequential experience may trigger a non-random response of adult beetles to hairy and
334 glabrous diets. While adult beetles fed on both hairy and glabrous leaves at similar
335 levels under no-choice conditions, larvae fed on hairy diets grew slower than those on
336 glabrous diets. This indicates that hairy diets are suboptimal for *P. brassicae*.

337 The foraging behaviors of *P. brassicae* were then modeled to reveal the
338 mechanism underlying the pattern of leaf damage to hairy and glabrous plants. Given
339 that *P. brassicae* is flightless and incapable of selecting plants among patches, the
340 optimal diet choice (Charnov 1976) is assumed to be a mechanism by which rare
341 suboptimal diets (i.e., hairy leaves) are less consumed under the small enclosed
342 condition. My collaborators and I modified the original model of optimal diet choice to
343 consider imperfect host recognition by *P. brassicae*, and then fitted the model to the
344 laboratory experiment data of leaf damage under the contrasting frequency of hairy and
345 glabrous plants (Sato *et al*. 2017). As a result of the parameter estimation, it was
346 estimated that decreased energy intake and increased handling time for the leaf beetles
347 accounted for the frequency-dependent damage on hairy plants (Fig. 2c). Hairy plants
348 were less damaged when glabrous plants became abundant, indicating associational
349 resistance for hairy plants. Contrarily, damages to glabrous plants weakly depended on
350 the frequency of two plant morphs. These findings indicate that the optimal diet choice
351 by *P. brassicae* can be a determinant of the pattern of AE between hairy and glabrous
352 plants.
353
354 *2-2. Manipulative experiment showing a rarity advantage in plant defense and fitness*
355 Even if herbivores feed on hairy and glabrous plants in a frequency-dependent manner,
356 it is still unknown whether this candidate mechanism leads to frequency-dependent
357 selection on the trichome dimorphism. Specifically with regard to the brassica leaf
358 beetle *P. brassicae*, it seems unlikely that herbivore preference alone generates negative
359 frequency-dependent selection, as they did not avoid rare glabrous diets (Sato *et al*.
360 2014: Fig. 3a). However, game theoretical models suggest that, if plants have defense

361 traits at the cost of their growth or fecundity, the benefit from associational effects
362 allows defended and undefended plants to coexist (Augner *et al*. 1991). This hypothesis
363 was tested using a laboratory and semi-field experiment manipulating the morph
364 frequency and presence/absence of *P. brassicae* (Fig. 2b; Sato & Kudoh 2016b; Sato &
365 Kudoh 2017b).

366 Figure 3 shows a schematic explanation of a mechanism by which conspecific
367 AE could be a mechanism for the maintenance of trichome dimorphism. Consistent with
368 the feeding preference assay (Sato *et al*. 2014), hairy plants were less damaged than
369 glabrous plants only when the hairy ones were rare (Fig. 3a: Sato & Kudoh 2016b; Sato
370 & Kudoh 2017b). This explains the rarity advantage for hairy plants in defense and
371 fitness (Fig. 3a, c). At the end of the experiments, both the hairy and glabrous plants had
372 a rarity advantage in fitness in the presence of beetles (Fig. 3c: Sato & Kudoh 2016b;
373 Sato & Kudoh 2017b), while in the absence of herbivory the glabrous plants had a
374 higher growth and fecundity (Fig. 3b: Sato & Kudoh 2016b; Sato & Kudoh 2017b).
375 Because the benefit of associational effects to hairy plants no longer exists under the
376 hairy-abundant condition (Fig. 3a), the cost of defense can be detected when hairy
377 plants become abundant (Fig. 3c). Of note, these fitness consequences were consistently
378 observed for both sexual and asexual reproduction, namely the number of flowers and
379 clones, of *A. halleri* (Sato & Kudoh 2017b). These experimental findings indicate that,
380 together with the defense-growth tradeoff (Fig. 3c), conspecific AE against the leaf
381 beetle (Fig. 3a) lead to negative frequency-dependent selection between hairy and
382 glabrous plants (Fig. 3b) (Sato & Kudoh 2017b).
383

384 *2-3. Field patterns of herbivory and polymorphism dynamics*

385 The set of experiments illustrated conspecific AE that can maintain the trichome
386 dimorphism of *A. halleri*, but it is still unknown whether these experimental findings
387 represent AE in the wild. Next, field observations on plant damage and demography
388 were conducted in a natural population in which the brassica leaf beetle *P. brassicae*
389 was a major herbivorous insect on *A. halleri* (Fig. 2b). Because *P. brassicae* is flightless
390 and the *A. halleri* population has a fragmented structure, AE is assumed to occur on a
391 small spatial scale. Thus, my collaborators and I tracked the aggregated structure of the
392 plants (referred to hereafter as “patch”) for four years in the field.

393 Hairy plants received less damage as the frequency of glabrous plants increased
394 in a series of plant patches (Sato *et al*. 2014; Sato & Kudoh 2017b). In contrast, leaf
395 damage on glabrous plants showed no significant and consistent relationship with the
396 frequency of glabrous plants in a patch (Sato *et al*. 2014; Sato & Kudoh 2017b). This
397 pattern of leaf damage was consistent with the results of a feeding assay. It was also
398 noteworthy that the relative fitness advantage of hairy plants as shown by flower
399 production in the presence of beetles increased with increasing frequencies of glabrous
400 plants (Sato & Kudoh 2017b). This pattern in the flower production agrees with the
401 manipulative experiment detecting negative frequency-dependent selection only in the
402 presence of *P. brassicae* (Fig. 3c). These findings support the relevance of
403 frequency-dependent herbivory and selection in the natural population.

404 A multi-year field survey also found that rarer morphs increased in frequency
405 in a series of plant patches over time (Sato & Kudoh 2017b). Statistical modeling was
406 used to reveal the mechanisms underlying the temporal dynamics of morph frequency in
407 the field (Sato *et al*. 2017). Based on the aforementioned theory of optimal diet choice,
408 my collaborators and I have modelled the effects of local-scale herbivory and spatial

409 structure on plant demography and then fitted the model to the observed changes in the
410 number of hairy and glabrous plants over four years. Based on a model selection
411 procedure, it was revealed that optimal diet choice by *P. brassicae* contributes to the
412 polymorphism dynamics observed in the field (Sato *et al*. 2017). The estimated
413 magnitude of interpatch dispersal suggested that long-distance seed dispersal was rare
414 and clonal reproduction largely contributed to the plant population growth. According to
415 the estimated parameters of antiherbivore defense, rarer morphs had higher fitness and
416 increased in frequency over time when the herbivore diet choice and cost of plant
417 defense were both involved (Fig. 2c). This joint approach using the optimality modeling
418 and field data strongly supports the hypothesis that AE against the leaf beetle promotes
419 the maintenance of the trichome dimorphism via negative frequency-dependent
420 selection.

421 In contrast to the constant density in the manipulative experiment (Fig. 3), not
422 only frequency but also the number of plants varies among field patches. However, the
423 effects of plant density on damage were much less significant than that of frequency,
424 and its magnitude and direction were inconsistent among the study years (Sato *et al*.
425 2014; Sato & Kudoh 2017b). The resource concentration or delusion of *A. halleri* for *P.*
426 *brassicae* was tested in more detail using the field data on the leaf damage and number
427 of *A. halleri* plants (Sato *et al*. 2017). If these two effects of plant density occur, there is
428 a non-linear relationship between the total amount of herbivory load and the total
429 number of plants within patches (Hambäck *et al*. 2014). In *A. halleri*, however, the total
430 amount of leaf damage increased linearly with the total number of plants in a patch
431 (Sato *et al.* 2017), presumably because the plant density in the field was too low to
432 reach a peak of resource loss by herbivory. Thus, at least for the temporal scales

observed, the field survey could not detect a significant role of plant density in AE against the leaf beetles.

Overall, the multiple lines of evidence have shown that conspecific AE mediated by *P. brassicae* lead to negative frequency-dependent selection and thereby maintain the trichome dimorphism in a natural *A. halleri* population. If hairy plants are rare, they can escape from leaf beetles and increase in frequency. On the other hand, if glabrous plants become rare, they outcompete hairy plants without any costs of trichomes. Consequently, this negative feedback loop prevents rare morphs from going extinct (Fig. 4). To date, negative frequency-dependent selection has been reported for plant species interacting with pollinators (Gigord *et al*. 2001) and pathogens (Antonovics & Ellstrand 1984; Brunet & Munht 2000), but it has not yet been demonstrated in plant defense against herbivores. The case study on *A. halleri-P. brassicae* interaction provides the first example to illustrate that conspecific AE drive negative frequency-dependent selection on an antiherbivore defense trait.

3. Perspective

Genetic polymorphisms are commonly observed in plant defense traits (Hughes 1991; Elle *et al*. 1999; Wise *et al*. 2009; Bernhardsson *et al*. 2013) and not limited to *Arabidopsis* trichomes. For example, the genetic basis of cyanogenesis in white clover *Trifolium repens* (Fabaceae) is well studied (Hughes 1991). Theory related to herbivore-mediated frequency-dependent selection was applied to geographical variation in a cyanogenesis polymorphism (Till-Bottraud & Gouyon 1992), but the existence of frequency-dependent selection has not been tested experimentally. Dimorphism of the glandular (sticky) and non-glandular (velvety) *Datura wrightii*

(Solanaceae) is inherited in the Mendelian fashion (van Dam *et al*. 1999). This chemical defense utilizing glandular exudates is costly for plant fitness (Elle *et al*. 1999), but it remains unknown why these sticky and velvety plants can coexist (Elle & Hare 2000). The genetic basis of glucosinolates, a major secondary metabolite of Brassicales, has been intensively studied. Genetic polymorphisms are observed in physiological pathways in glucosinolate biosynthesis and result in diverse profiles among natural *Arabidopsis* accessions (Chan *et al*. 2010). Conspecific AE have not yet been examined in these defense traits but could provide insights into mechanisms for the maintenance of antiherbivore defense polymorphism within a plant population.

We should note here that not only AE but also other factors may shape genetic variation in antiherbivore defenses. It has been hypothesized that genetic variation may be maintained by spatiotemporal fluctuation in selection pressure and stochastic gene flow among populations (e.g., Levene 1953; Turelli *et al*. 2001; but see Spichtig & Kawecki 2004). The present paper has focused on local-scale AE so far, but to what spatial and temporal scales can AE contribute to the maintenance of polymorphism in plant defense? In the final chapter, I discuss a spatial and temporal perspective of conspecific AE and the maintenance of plant defense polymorphism. Concerning the spatial scale, several researchers discussed interspecific AE at a landscape scale (reviewed by Barbosa *et al*. 2009). The geographic mosaic of coevolution (Thompson 2005) proposes a landscape perspective of plant–insect interactions, and this spatial point of view is applied to geographic variation in plant defense (e.g., Berenbaum & Zangerl 1998; Zangerl & Berenbaum 2003; Toju *et al*. 2011; Bernhardsson *et al*. 2013). In particular to temporal dynamics, the concept of eco-evolutionary dynamics has recently suggested interactions between plant genetic variation and herbivore abundance

(Turcotte 2011; Turcotte *et al*. 2013; Agrawal *et al*. 2013). Because not only frequency- but also density-dependent selection is a key aspect of AE (Table 1), conspecific AE may provide insights into how rapid evolution in plant defenses affects the population dynamics of plants and herbivores.

3-1. Can AE create a source population for geographical variation in plant defense?

Existing theory suggests that negative frequency-dependent selection contributes to geographic cline in a polymorphism, as it makes morph frequency intermediate rather than expected by the results of disruptive selection alone (Clarke 1966; Endler 1977; Till-Bottraud & Gouyon 1992). Till-Bottraud & Gouyon (1992) applied this prediction to explain a cyanogenesis polymorphism along an altitudinal cline in white clover. However, herbivory regimes usually have more complex spatial patterns than a cline, involving differences in herbivore species (Hare & Elle 2002) and the presence of other host plants (Zangerl & Berenbaum 2003). Thus, plant–herbivore interactions are expected to result in complex geographic variation rather than a simple cline in a plant defense polymorphism.

The geographic mosaic of coevolution proposes a conceptual framework for investigating the complex metapopulation structure of local adaptation/maladaptation (Thompson 2005). Specifically, the theory of geographic mosaic of coevolving polymorphism hypothesizes that disruptive and balancing selection jointly shape complex patterns of host defense polymorphism (Thompson 2005). In plant–herbivore interactions, Berenbaum and Zangerl (1998) presumed that intense attacks by parsnip webworm *Depressaria pastinacella* on abundant chemotypes of a wild parsnip *Pastinaca sativa* (Apiaceae) led to negative frequency-dependent selection and thus

505 favored the intermediate chemotype frequency. Given that AE should occur among
506 individual plants in close vicinity, this may alter spatial patterns of plant defense only at
507 a local scale. More generally, the metapopulation framework assumes the existence of
508 source and sink populations of local adaptation (Thompson 2005). When AE favors
509 polymorphism within a local population, this polymorphic population could be a source
510 of polymorphism on a metapopulation scale via gene flow (Fig. 5).

511 Indeed, herbivore regimes and frequencies of hairy and glabrous plants vary
512 among natural populations of *Arabidopsis halleri* subsp. *gemmifera*, where AE do not
513 occur against all herbivorous insects on *A. halleri*. Sato and Kudoh (2015) found that
514 AE were detectable when *A. halleri* interacted with slow-moving herbivores such as *P.*
515 *brassicae*. AE tend to be obscured when plants are attacked by flying herbivores such as
516 the green-veined white butterfly *Pieris napi* and cabbage sawfly *Athalia infumata*,
517 although trichomes themselves can prevent herbivory by these herbivores (Sato &
518 Kudoh 2015). Field surveys revealed that frequencies of glabrous plants declined as
519 herbivory pressure became intense (Sato & Kudoh 2017a). Hairy-monomorphic
520 populations were discovered where white butterflies were frequently observed. Despite
521 experiencing the severest herbivory pressure, however, a population where *P. brassicae*
522 was predominant still had an intermediate morph frequency (Sato & Kudoh 2017a).
523 Furthermore, a proximate population was also polymorphic despite the prevalence of
524 white butterflies (Sato & Kudoh 2017a). These observations support the hypothesis that
525 herbivore-mediated frequency-dependent selection sustains polymorphism, which can
526 be a source of genetic variation in proximate populations (Fig. 5b). Further questions
527 remain about how conspecific AE contributes to the maintenance of polymorphism at
528 the metapopulation level.

529

530 *3-2. Do AE affect population dynamics via the maintenance of defense polymorphism?*

531 A growing number of studies have shown that herbivores drive the evolution of plant
532 defense on ecological time scales (Zangerl & Berenbaum 2008; Agrawal *et al*. 2012;
533 Fukano & Yahara 2012; Sakata *et al*. 2014). Recent studies have also shown that such
534 evolutionary changes in a host plant species alter the population dynamics and
535 community structures of herbivores (Crutsinger *et al*. 2006; Johnson *et al*. 2006;
536 Turcotte *et al*. 2011; Agrawal *et al*. 2013), which may in turn affect plant density. Now
537 that conspecific AE is known to maintain polymorphism in plant defense, it is possible
538 that such a sustained polymorphism affects eco-evolutionary dynamics in
539 plant–herbivore interactions. In the context of AE, Underwood *et al*. (2014) advocate
540 that manipulating both the density and frequency of multiple plant species is important
541 for complete understanding of the long-term coexistence of multiple plant species under
542 AE (as introduced in Section 1-2.). When this notion is applied to polymorphisms
543 within a plant species, AE may play an important role not only through frequency- but
544 also through density-dependent selection in plant defense.

545 Once the mechanisms of frequency- and/or density-dependent selection are
546 experimentally shown, it is possible to estimate the probability of coexistence from the
547 experimental data (e.g., Damgaard 1998; Inouye 2001; Miller & Rudgers 2014) or to
548 predict temporal dynamics in the field (Kim *et al*. 2013; Le Rouzic *et al*. 2015) based on
549 a given theoretical model. This modeling approach, combined with empirical data, will
550 be a powerful tool to reveal how AE contribute to eco-evolutionary dynamics in
551 plant–herbivore interactions. For example, Sato *et al*. (2017) took such a modeling
552 approach to *A. halleri–P. brassicae* interactions although the modeling was limited in

553 predicting long-term interactions between the evolution of plant defenses and herbivore
554 population dynamics. Specifically, the magnitude of herbivory in the current year was
555 not clearly correlated with that in the previous year or with the frequency of hairy plants
556 (Sato *et al*. 2017). If long-term data on herbivory and population dynamics are obtained,
557 the modeling approach might enable us to predict how the existence of polymorphism
558 affects herbivory pressure and in turn alters the morph frequency within a plant species.
559
560 *3-3. Concluding remarks*
561 Associational effects, or neighbor effects, are remarkable phenomena in plant–herbivore
562 interaction, but evidence for conspecific AE is still limited. By focusing on polymorphic
563 defense traits within a plant species, the present review highlights the potential
564 importance of conspecific AE in maintaining polymorphism in an antiherbivore defense
565 trait. The multiple lines of evidence from *P. brassicae–A. halleri* interaction show that
566 conspecific AE maintains antiherbivore defense polymorphism via negative
567 frequency-dependent selection. Future studies should focus on the stabilizing role of AE
568 in genetic variation in plant defense, as diverse plant species possess considerable
569 variation in terms of antiherbivore defense traits.
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912 **Table 1.** Examples of associational effects that cause frequency- and/or density-dependent herbivory between plant species/genotypes.

Factor	Article	Study system		Neighborhood composition
		Plant (or Resource)	Herbivore (or Consumer)	
Density	Rand (2003)	*Atriplex patula* and *Salicornia europaea*	Polyphagous beetle *Erynephala maritima*	Presence/absence of the other species with low/high density
	Russell & Louda (2005)	*Cirsium canescens* and *C. undulatum*	Florivorous weevil *Rhinocyllus conicus*	Density of the other species in natural populations
Frequency	Bergvall *et al*. (2006)	Low- and high-tannin food	Fallow deer *Dama dama*	Rare or abundant type A against B (= 2 frequency conditions)
	Wise *et al*. (2009)	Candy-cane and erect-stem genotype of *Solidago altissima*	Oligophagous gall-fly *Eurosta solidaginis*	Rare or abundant type A against B (= 2 frequency conditions)
	Sato & Kudoh (2016b)	Hairy and glabrous genotype of *Arabidopsis halleri* subsp. *gemmifera*	Oligophagous beetle *Phaedon brassicae*	Rare or abundant type A against B (= 2 frequency conditions)
	Garrido *et al*. (2016)	Resistant and tolerent genotype of *Datura stramonium*	Oligophagous beetle *Lema daturaphila*	Rare, even, and abundant type A against type B (= 3 frequency conditions)
Both	Kim & Underwood (2015)	*Solidago altissima* and *Solanum carolinense*	(incl. multiple species)	Response surface design of 4 frequency and 4 density conditions
	Hahn & Orrock (2016)	*Solidago nemoralis* and *S. odora*	Grasshoppers	Rare, even, and abundant type A against type B (= 3 frequency conditions) at high/low densities.
	Verschut *et al*. (2016)	Balsamic vinegar solutions	*Drosophila melanogaster*	Different resource concentration (2 levels) with 3 or 5 frequency conditions

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Figure legends

Figure 1. Stepwise approach to reveal causes and consequences of associational effects (AE) on plant coexistence. The reference indicates studies comprising a focal approach. Details of the selected references are shown in Appendix.

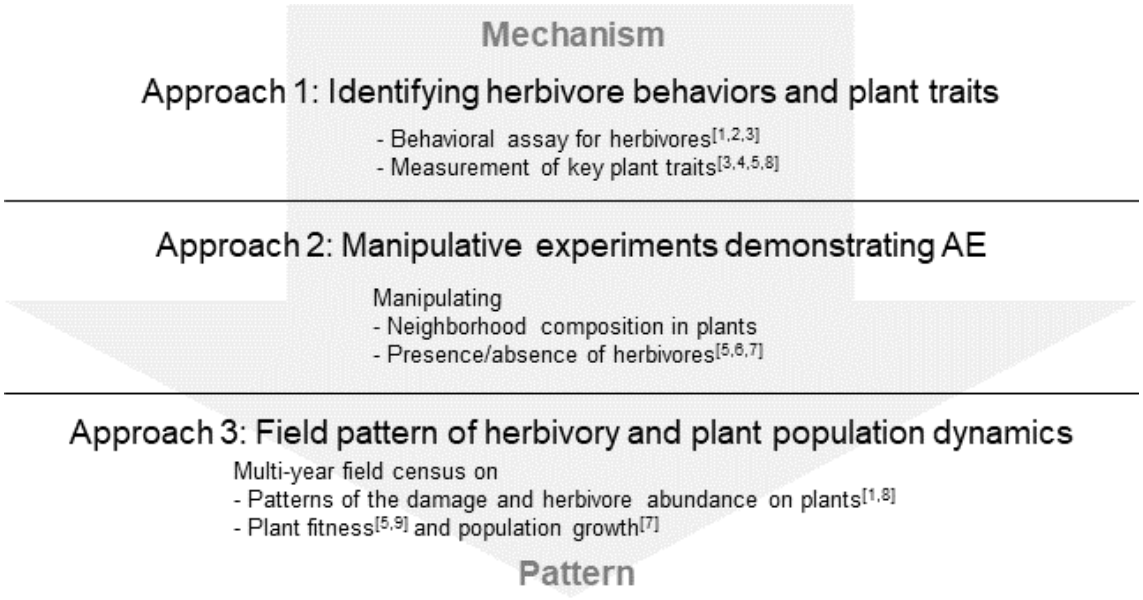
Figure 2. Comprehensive test for the maintenance of the trichome dimorphism of *Arabidopsis halleri* subsp. *gemmifera* mediated by associational effects against a leaf beetle *Phaedon brassicae*. **(a)** Photographs of plants and insects; **(b)** Empirical approaches from laboratory experiments to field observation; **(c)** Modeling herbivory, plant fitness, and polymorphism dynamics. Insets in the panel **(c)** are modified from Sato *et al*. (2017).

Figure 3. Mechanism through which associational effects and a cost of defense jointly cause negative frequency-dependent selection between hairy and glabrous plants. Panels **(a)**, **(b)** and **(c)** represent differences in herbivory, intrinsic fitness, and realized fitness between hair and glabrous plants, respectively. H > G and H < G indicates hairy-abundant and glabrous-abundant condition, respectively.

Figure 4. Evolutionary dynamics wherein the trichome dimorphism of *Arabidopsis halleri* subsp. *gemmifera* can be maintained by the herbivore-mediated frequency-dependent selection. Frequency-dependent feeding preference by herbivores and plant defense-growth tradeoff results in a negative feedback loop that allows rarer plant morphs to invade into a population.

Figure 5. Specific hypothesis for how local-scale associational effects (AE) contribute to geographic variation in plant defense via the maintenance of source population in a polymorphism. **(a)** Relationship between the herbivory pressure and morph-frequency in the presence (solid line) and absence (dashed line) of AE. **(b)** Contribution of a source polymorphic population created by AE to the maintenance of polymorphism at a metapopulation scale. Grey and white square represents the region of intense and moderate herbivory, respectively. White arrows represent gene flow from a source to nearby populations.

946 **Figure 1**



[1] Tahvanainen & Root (1972); [2] White & Whitham (2000); [3] Bergvall *et al.* (2006); [4] Barton & Browers (2006); [5] Agrawal (2004); [6] Rand (2003); [7] Callaway *et al.* (2005); [8] Russell & Louda (2005); [9] Karban & Maron (2002)

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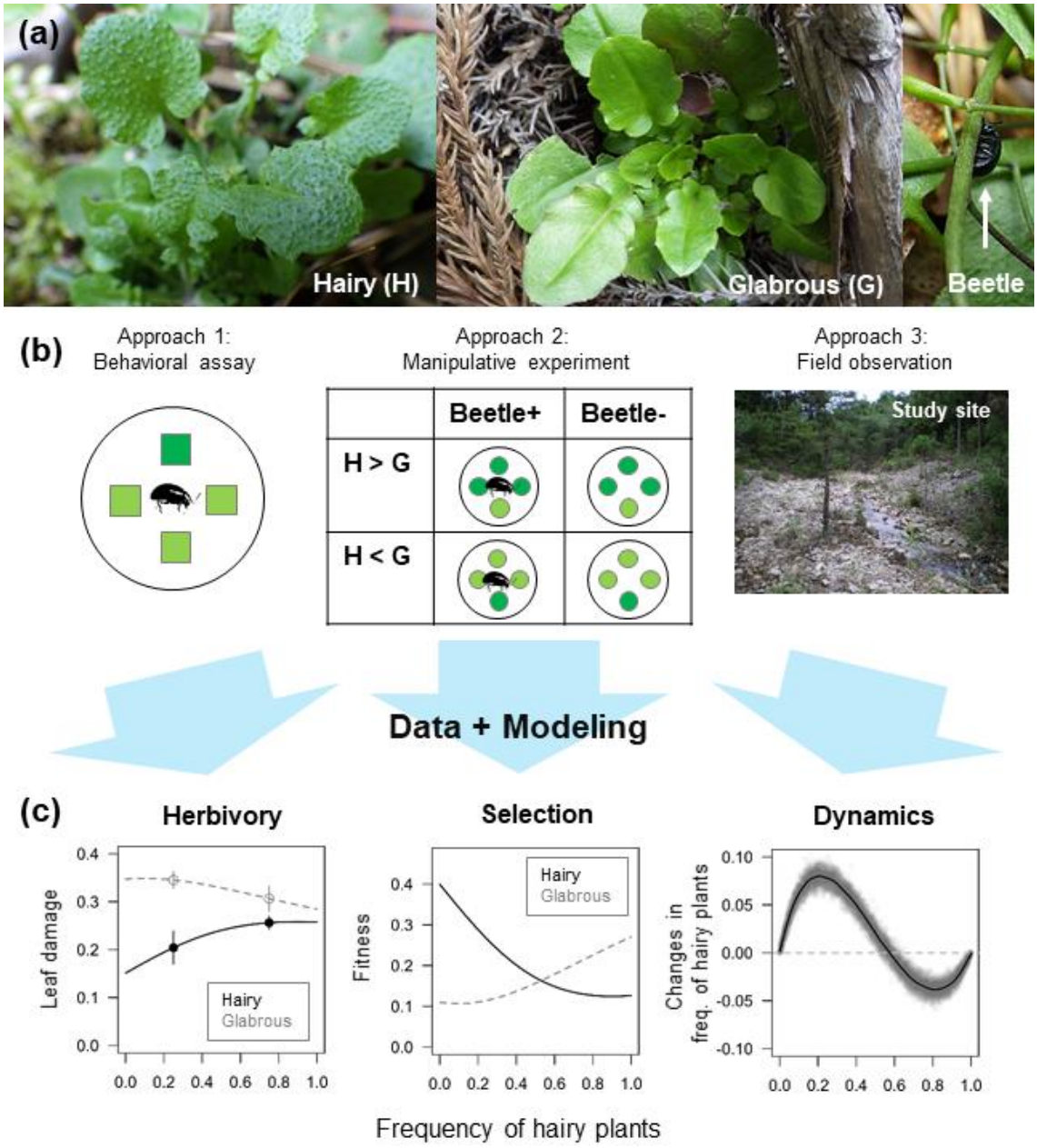
961 **Figure 2**



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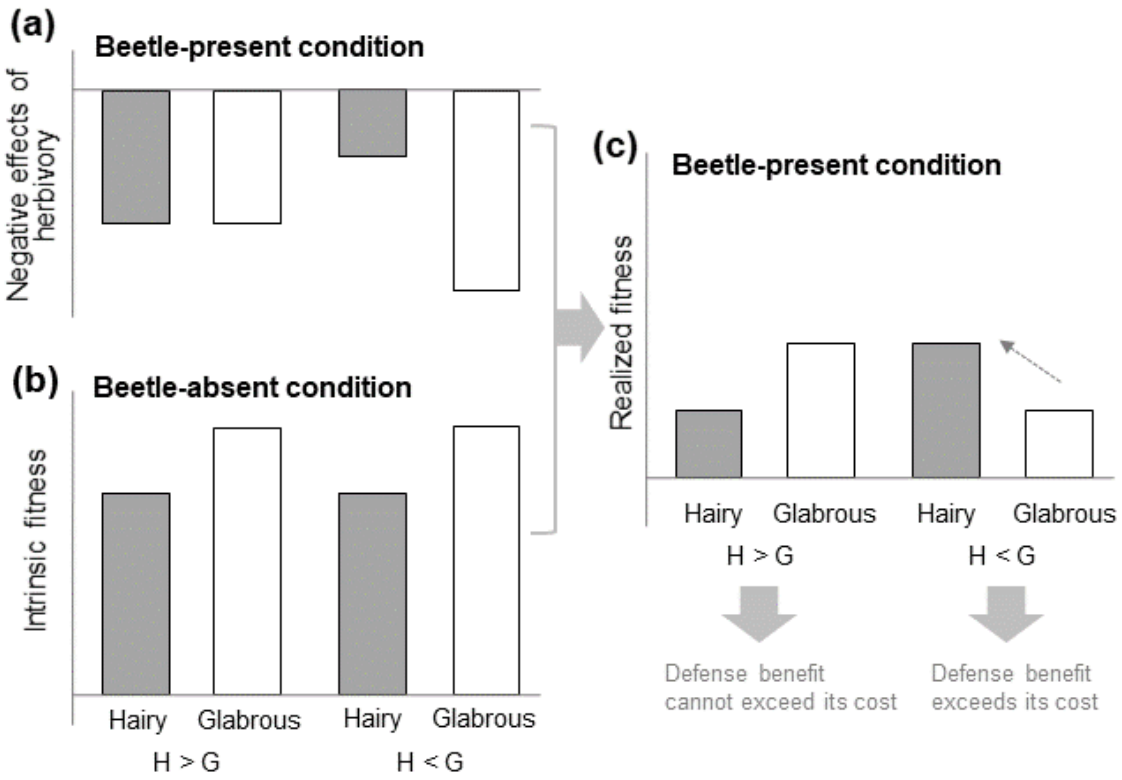
968 **Figure 3**



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982 **Figure 4**

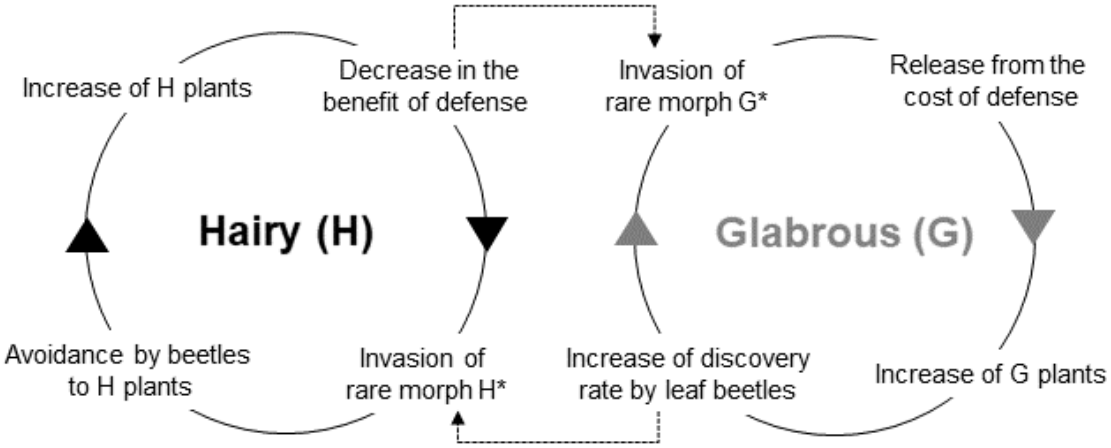

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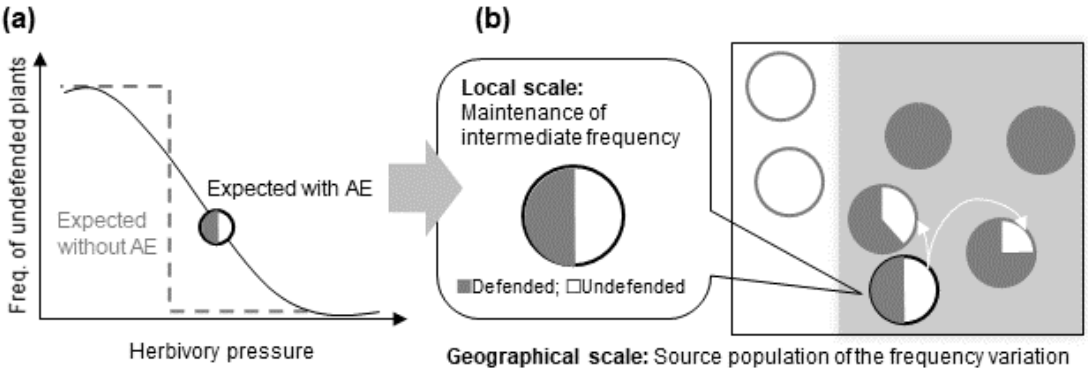
Figure 5



Appendix. Selected examples of associational effects in plant-herbivore interaction. Study system (plants and herbivores), design (experimental design and spatial scales, reciprocal data or not), and results (measurement, outcome, and putative mechanism) are summarized.

Reference	Study system			Study design			Results		
	Herbivore	Plant species	Plant traits	Experimental design	Scale	Reciprocal or not	Measurement	Outcome	Mechanism
Tahvanainen & Root (1972)	A specialist flea beetle (*Phyllotreta cruciferae*)	*Brassica oleracea* and tomato or tobacco	Odor (shown by a choice assay)	Monoculture of *B. oleracea* and polyculture of three species. The tomato and tobacco was added to the equal density of *B. oleracea* in the polyculture.	Within-patch	NA for tomato and tobacco	The number of beetles per plant and plant biomass for *B. oleracea*	AR: The number of beetles per plant was higher, and plant biomass decreased under the monoculture.	Odor cues for herbivores
Rausher (1981)	A specialist butterfly (*Battus philenor*)	*Aristolochia reticulata* and others species in near vegetation	Unidentified	0.5 m-radius vegetation surrounding an *A. reticulata* individual was removed. Non-removed patches were assigned to the control.	Within-patch	NA for counterparts	Oviposition per *A. reticulata* plant	AR: Isolated plants suffer more oviposition, butterflies spend more time in the isolated patch.	Searching effeciency of herbivores
Risch (1981)	Mono- Oligo-, Polyphagous beetles (6 species)	Corn (*Zea mays*), beans (*Phaseolus vulgaris*), and squash (*Cucurbita maxima*)	Unidentified	Intercropping one crops into another; monoculture, diculture triculture for 3 species (= 7 conditions)	Within-patch	Reciprocal	No of beetles per plant	AR: The beetle abundance reduced under polycultic conditions except for a squash-bean diculture	Resource concentration (plant density)
Hay (1986)	Generalist fishes	Multiple species of Marine algae	Unidentified	Solitary or paired culture in a closed cage	Within-patch	Reciprocal	Within-patch: predation risk by fishes	AR for palatable algae to generalist fishes	Physical attachments to unpalatable algae
White & Whitham (2000)	Fall cankerworm *(Alsophila pometaria*).	Cotton (*Populus angustifolia* × *P. fremontii*), and box elder (*Acer negundo*)	Unknown, (but a choice test showed that the cankerworm prefer to the box elder).	Transplanting juvenile cottons to three conditions: Nearby box elder, Nearby matured cotton, Stand alone.	Within-patch	NA for the box elder	Larvae density per shoot of the juvenile cotton	AS: Cotton suffered from the higher density nearby a heterospecific counterpart than nearby conspecifics or stand alone.	Herbivore dispersal from palatable to unpalatable plants
Karban & Maron (2002)	Generalist grass-hoppers (incl. multiple species)	Sagebrush (*Artemisia tridentata*) and tobacco (*Nicotiana attenuata*)	Volatile chemical	Tobacco was transferred near damaged or intact sagebrush.	Within-patch	NA for Sagebrush	The number of flowers and capsules per tobacco	AR: tobacco produced more flowers and capsules nearby damaged sagebrush.	Volatile communication between plants
Hambäck *et al.* (2000)	A specialist leaf beetle (*Galerucella calmariensis*)	An aromatic shrub (*Myrica gale*) and a perrenial herb (*Lythrum salicaria*)	Unidentified	Stand-alone or within-*M. gale* patch condition for *L. salicaria*	Within-patch	NA for *M. gale*	Oviposition, leaf damage, seed production per individual *L. salicaria*.	AR: *L. salicaria* incurred less oviposition and damage, and produce more seeds nearby *M. gale*	Odor or vidual cypsis. Predator abundance did not differ between the two conditions.
Rand (2003)	A generalist leaf beetle (*Erynephalam aritima*)	Two forbs, *Atriplex patula* var. *hastata* and *Salicorniae uropaea*	Unidentified (but feeding preference was shown by a choice test).	The presence/absence of herbivores for 3 conditions: *Atriplex* alone with low density, *Atriplex* alone with high density, *Atriplex* with *Salicornia*	Within-patch	NA for *Salicornia*	Leaf damage, the number of larvae, and survival (%) of plants.	AS: *Atriplex* suffered more leaf damage and less survival nearby *Salicornia*	Diet-mixing by generalist herbivore
Agrawal (2004)	A specialist beetle (*Tetraopes tetraophthalmus*)	a milkweed (*Asclepias syriaca*) and a grass (*Liriomyza asclepiadis*)	Trichome, Latex, Nitrogen contents	Grasses or beetles were added to a potted milkweed: 4 treatments of Control, Grass+, Beetle+, and Grass&Beetle+. Grass alone, Grass&Milk&Beetle, Grass&Milk was also set.	Within-patch	Grass biomass data: Grass alone = Grass&Milk&Beetle > Grass&Milk	Milkweed biomass, fruit production, fruit mass; and grass biomass	AS: milkweed biomass and fecundity was Control ≒ Grass+ > Beetle+ > Grass&Beetle+.	Dispersal from primary host plants to secondary ones.

Callaway *et al.* (2005)	Mammals	Four unpalatable herbs (*Cirsium* , *Veratrum, Luzula, Alchemilla*) and two palatable herbs (*Anthoxanthum* , *Phleum*)	Spines (for *Cirsium*) or toxin (for *Veratrum*)	Four focal species × within/outside grazing fence × exclusion of *Cirsium* or *Veratrum* = 24 treatments	Within-patch	NA for *Cirsium* and *Veratrum*	Annual growth rates of four plant species.	AR: increased growth rate of two palatable species, no increase of growth rate of unpalatable two species nearby *Cirsium* and *Veratrum*	avoidance by grazers to plant spines and toxins.
Russell & Louda (2005)	Florivorous weevil, *Rhinocyllus conicus*	Two congeneric natives, *Cirsium canescens* and *C. undulatum*	Plant phenology	> 10-yrs field census in 13 sites where the two plant species co-occur	Within-site	NA for *C. canescens*	Proportion of weevils and their eggs on *C. undulatum* .	AR for *C. undulatum* : The proportion of weevils and eggs was negatively correlated with the number of *C. canescens.*	Phenological syncronicity of flowering between the two species.
Bergvall *et al.* (2006)	Fallow deer (*Dama dama*)	Unknown. Synthetic food containing different tannin levels	Chemical (Tannin)	Bad-patch and good-patch contains 7:1 and 1:7 of high:low tannin buckets, respectively. Single- and group-foraging of deers were also tested.	Within and between patch	Reciprocal	Log-consumption per bucket (g).	AS for low-tannin buckets: More consumption on low-tannin buckets in the bad-patch than those in the good-patch. AR for high-tannin buckets: Less consumption on high-tannin buckets in the good-patch than those in the bad-	within- and among-patch foraging behavior of mammalian herbivores.
Barton & Bowers (2006)	None (only defense phenotypes were evaluated)	Interspecific: two congeners, *Plantago lanceolata* and *P. major*	Chemical (Iridoid glucoside)	2 species × (conspecific, heterospecific, no neighbor) × 2 harvesting time points = 12 conditions	Within-patch	Reciprocal	Within-patch: % Dry Weight of Iridoid Glycosides	AS for *P. lanceolata* ?: *P. lanceolata* surrounded by heterospecific plants produced less defensive chemicals compared to those surrounded by conspecifics.	Plant trait change via direct competition between plants.
Le Guigo *et al.* (2012)	A specialist aphid (*Brevicoryne brassicae*) and generalist aphid (*Myzus persicae*)	Three *Brassica* species and tomato (*Solanum lycopersicum*)	Chemical (glucosinolate?)	A *Brassicae oleracea* individual was surrounded by 6 conspecific or heterospecific individuals (= 7 treatments in total).	Within-patch	Reciprocal	The number of aphids on a focal *B. oleracea,* compared between conspecific and interspecific conditions	AS when a herbivore is specialist; AR when a herbivore is generalist.	Chemical attraction or repulsion
Castagneyrol *et al.* (2013)	Leaf-chewers (beetles, caterpillars, and grasshoppers) or Leaf-miners (incl. 5 species)	*Betula pendula* , *Quercus robur* , *Quercus pyrenaica* , *Quercus ilex* , *Pinus pinaster*	Plant height, and leaf area, N and P contents, leaf life span	31 possible mono- and poly-culture combinations of 1~5 plant species	Within/Between patch	Relative values of two species (for plant height data)	Leaf area loss (by chewers) and leaf-miner abundance at plot and individual scale.	AR against leaf-miners: no AR against leaf-chewers	Variation in leaf traits and apparency (plant height)
Kim & Underwood (2015)	3 species of specialists (for *S. carolinense*), and 3 species of generalist herbivores	*Solanum carolinense*, *Solidago altissima*	Unidentified	Response surface design (1, 6, 12, 18 total plant number with 4 frequencies of *S. altissima*)	Within-patch	Reciprocal (shown in the supplement)	Leaf damage (%) of individual plants	AS for *S. carolinense and* damage was positively density-dependent.	Unknown (but those depending on the plant density and frequency)

Abbreviation: AR; Associational resistance, AS; Associational susceptibility, NA; No information available